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Please send me:

1) Hawkins et al.
"Adapting antibodies for clinical use-monoclonal antibody engineering technology: a review"
BR. MED. J.
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2) Obrist etal
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3) Reisfeld et al.
"Involvement of B lymphocytes in the growth inhibition of human pulmonary melanoma metastases in ..."
CANCER RESEARCH
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1996

4) Challita et al.
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5) Reisfeld et al.
"Immunocytokines: a new approach to immunotherapy of melanoma"
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"Characterization of a RANTES anti-HER2/neu antibody fusion protein for cancer immunotherapy"
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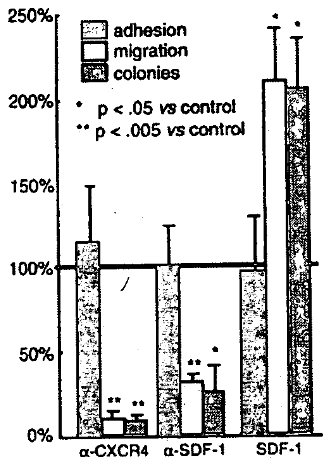
Vol. 92 (10 suppl. 1 part 1-2) p.24A.

Abstract# 88 **Poster Board#/Session: 88-I**

SDF-1 AND ITS RECEPTOR CXCR4 ARE REQUIRED FOR MIGRATION OF HUMAN B CELL PRECURSORS UNDER STROMA AND THEIR PROLIFERATION IN CULTURE.

Karl Scheidweiler*, Ion Ritterman*, Jihong Tang*, Eric Fedyk*, Timothy Springer, Daniel Ryan. *Univ. of Rochester and Harvard Medical Schools, Rochester, NY, Boston, MA, USA.*

Human B cell precursors migrate underneath bone marrow stromal cells and require stromal contact for optimal *in vitro* proliferation. The signals regulating this migratory process are unknown, nor is it clear whether actual migration, or just adhesion alone, is necessary for proliferation of B cell precursors. Of multiple chemokines tested by RT-PCR, human bone marrow stroma cultured under lymphoid supportive conditions expressed only SDF-1 and MCP-1. We assessed the effect of blocking anti-SDF-1, anti-MCP-1, anti-CXCR4 (SDF-1 receptor), and soluble SDF-1, on simple adhesion, migration and proliferation of human B cell precursors. Adhesion and migration were assessed using NALM6 and Reh lines, while proliferation of CD34+ B cell precursors was assessed using a stromal-dependent TdT+ colony assay. Anti-SDF-1 and anti-CXCR4 blocked both migration and proliferation, but not adhesion, of human B cell precursors (see figure; values given as percent of control ± SEM). Anti-MCP-1 had no effect on migration or proliferation. Normal human marrow B cell precursors expressed surface CXCR4 by flow cytometry using anti-CXCR4 MoAb. Unexpectedly, addition of exogenous SDF-1 (10-1000 ng/ml) increased both migration and proliferation of human B cell precursors, again without affecting adhesion. These results indicate that the chemokine-receptor pair SDF-1/CXCR4 is not essential for initial adhesion of human B cell precursors to bone marrow stroma, but is required for their subsequent migration. Modulation of migration by blocking antibodies or addition of chemokine has virtually identical effects on stromal-dependent B cell precursor colonies, suggesting that migration of B cell precursors into the stromal layer may be essential for their development.



Abstract# 89 **Poster Board#/Session: 89-I**

CHARACTERIZATION OF A RANTES ANTI-HER2/neu ANTIBODY FUSION PROTEIN FOR CANCER IMMUNOTHERAPY.

Pia M. Challita-Eid, Camille N. Abboud, Sherie L. Morrison, Shannon P. Hilchey, Manuel L. Penichet, Scott F. Rosebrough, Joseph D. Rosenblatt. *Department of Microbiology and Molecular Genetics, Molecular Biology Institute, UCLA, Los Angeles, CA; Hematology-Oncology Unit, Department of Medicine, University of Rochester Cancer Center, Rochester, NY, USA.*

The successful eradication of cancer cells in the setting of minimal residual disease may require targeting of metastatic tumor deposits that evade the immune system. We combined the targeting flexibility and specificity of monoclonal antibodies with the immune effector function of the chemokine RANTES to target established tumor deposits. We describe the construction of an antibody fusion molecule with variable domains directed against the tumor-associated antigen HER2/neu, linked to sequences encoding the chemokine RANTES (RANTES.her2.IgG3). RANTES is a potent chemoattractant of T cells, NK cells, monocytes and dendritic cells, and expression of RANTES has been shown to enhance immune responses against tumors in murine models. We show that RANTES.her2.IgG3 fusion protein binds specifically to HER2/neu antigen expressed on EL4 cells and on SKBR3 breast cancer cells as assayed by flow cytometry. RANTES.her2.IgG3 could elicit actin polymerization of THP-1 cells and transendothelial migration of primary T lymphocytes. RANTES.her2.IgG3 prebound to SKBR3 cells also facilitates migration of T-cells. In vivo studies in SCID mice demonstrate specific localization of RANTES.her2.IgG3 to HER2/neu expressing tumor cells within 24 hours of antibody administration. Modeling studies in immunocompetent as well as B-cell deficient mice are ongoing in the laboratory. RANTES.her2.IgG3, alone or in combination with other chemokine or cytokine fusion antibodies, may be a suitable reagent for recruitment and activation of an expanded repertoire of effector cells to tumor deposits.

Abstra **Poster Board#/Sessio**

MODULATION OF TRANSCRIPTION FACTOR ACTIVITY IMMATURE B-CELLS FOLLOWING CD38 LIGATION.

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CD38 is a transmembrane glycoprotein expressed at high levels in lym progenitor cells and in activated lymphocytes. Ligation of CD38 with the Ma (mouse anti CD38; IgG_1) stimulates growth of mature lymphocytes, howe several pre-B cell lines it is associated with inhibition of cell growth and ind of apoptosis which only manifests in the presence of bone marrow stromal ce stromal cell cytokines such as IL-7, IL-3 or SCF. Multiple studies have show ligation of CD38 results in tyrosine phosphorylation of syk, c-cbl phospholipase C-gamma and also activates phosphatidylinositol 3-kinase (PI To further understand the molecular mechanisms involved in the cellular resp of immature B cells we investigated modulation of transcription factor acti the human pre-B cell line Nalm-6. Electrophoretic mobility shift assays (E were performed using total cell lysates of 2 × 10^7 Nalm-6 cells treated minutes with mouse IgG1 ($mIgG_1$) (control), $mIgG_1$+IL-3, $mIgG_1$+IL-7 (T16), CD38+IL-3 and CD38+IL-7. Mabs, IL-3 and IL-7 were us concentrations of 10 μg/ml, 10 ng/ml and 25 ng/ml respectively. Constitu activated STAT-1, STAT-4 (weak), CREB and NFκ-B were present in Na cells growing exponentially. NFκ-B activation as measured by densito scanning of EMSA autoradiographs indicated increases (relative to the contr 30%, 28% and 51% induced by IL-3, IL-7 and CD38 respectively. Combina of CD38 with IL-3 or IL-7 had a negative effect on NF κ-B activation, the combination reducing activation to below constitutive levels. Similar analy STAT-1 activation showed increases of 25%, 39% and 107% induced by IL-7 and CD38 respectively. Once again combinations of CD38 with IL-3 or had a negative effect, the latter showing a 23% decrease relative to consti activation or a 130% decrease relative to activation by CD38 alone. No chang activation levels of CREB or STAT-4 were seen as a result of stimulation by IL-7, CD38 or combinations thereof. To the best of our knowledge activati STAT-1 and NFκ-B by CD38 ligation has not been previously reported interesting to speculate that the cellular responses induced by the combinati CD38 and stromal cell cytokines may be the result of signal diversion which activation of transcription factors such as STAT-1 and NFκ-B.

Abstract# 91 **Poster Board#/Sessio**

DIFFERENTIAL SIGNALING BY HUMAN AND MURINE STRO CELLS THAT SUPPORT HUMAN B CELL DEVELOPMENT.

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Hematopoietic stem cell (HSC) commitment and expansion into the B-li occurs in a microenvironment of stromal cells and extracellular components, but the identity and function of the critical factors required completely understood. The current study compared murine and human st cells for their capacity to support human HSC development into the B-li Two populations of human fetal bone marrow (BM) HSC were isolated b sorting; $CD34^+CD19^-$ HSC (a pool of HSC and common lymphoid progenito $CD34^+/CD10^-/CD19^-/CD45RA^-$ HSC (a population enriched for the primitive HSC, given the expression of CD45RA and CD10 myeloid/erythroid/lymphoid progenitors). HSC were plated onto non-irra murine S17 stromal cells, human fetal BM stromal cells (VCAM-1^+), or h skin fibroblasts (VCAM-1^-). In stromal cell cultures initiated with $CD34^+$ HSC, we found that greater numbers of $CD19^+$ B-lineage cells emerged foll 7-35 days in culture on human BM stromal cells and fibroblasts, compared t On day 21, 4.7-7.1 × 10^3 $CD19^+$ cells were present on human BM stromal c human fibroblasts, whereas <500 $CD19^+$ cells were detected on S17. Huma stromal cell or fibroblast cultures contained cells with a continuum of surface expression at days 7-14 as determined by mean fluorescent int (MFI). Day 7 MFI was 88 ± 17 and 85 ± 22 for human BM stromal cel human fibroblasts, respectively; and day 14 MFI was 203 ± 41 and 226 ± human BM stromal cells and human fibroblasts, respectively. In contrast, cel emerged on S17 had 3-4 fold higher levels of surface CD19 (282 ± 9 and 86 on day 7 and 14, respectively) and higher side scatter properties, compa $CD19^+$ B-lineage cells that emerged on human BM stromal cells or fibro Immature B cells expressing μ/κ or μ/λ B cell receptors (5-10% of $CD19^+$ were present by day 21 when $CD34^+CD19^-$ HSC were cultured on huma stromal cells or human fibroblasts. Notably, $CD19^+$ cells in S17 cultures d express cell surface BCR after 35 days. RT-PCR analysis of TdT and CD expression, and D_{HQ52}-J_H rearranged gene expression, indicated that comp levels and kinetics of induction of these genes occurred when $CD34^+CD19$ were plated on human or murine stromal cells. $CD34^+/CD10^-/CD19^-/CD$ HSC were less capable of developing into $CD19^+$ B-lineage cells on hum murine stromal cells. Interestingly, although CD19 was detected by RT-PCI days following plating of $CD34^+/CD10^-/CD19^-/CD45RA^-$ HSC on hum murine stromal cells, TdT was only expressed in human stromal cell cultur results demonstrate that human stromal cells support the development of B-l cells which manifest patterns of gene expression consonant with development in vivo. In contrast, human HSC cultured on murine S17 s cells yield a unique population of $CD19^+$ cells that, to our knowledge, ha been detected in vivo. These two stromal cell cultures can therefore